Discovery of a Mycoplasmalike Organism Associated with Diseased Soybeans in Mexico

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ABSTRACT

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Soybean plants in southwestern Mexico had symptoms similar to those reported for machismo disease in Colombia. Phloem of diseased plants displayed positive Dienes' staining. Electron microscopy revealed many mycoplasmalike organisms in sieve elements of diseased samples from Mexico and Colombia. Numerous leafhoppers (*Scaphytopius fuliginosus*), known vectors of the machismo disease agent, were found in affected Mexican fields. Machismo disease of soybeans appears to be more widely distributed than previously thought.

The severity of machismo disease of soybeans, first reported in the Cauca Valley of Colombia in 1968, dramatically increased until 1979, when as many as 80% of the plants in that area were affected and losses in some fields reached 1,600 kg/ha (7). On the basis of symptoms, transmission by the leafhopper *Scaphytopius fuliginosus* Osborne, and remission of symptoms after treatment of infected plants with tetracycline, Granada (7,8) suggested that the causal agent of the disease was a mycoplasmalike organism (MLO). Subsequent electron microscopic examination has revealed the presence of MLOs in the phloem of diseased, but not healthy, soybean plants (G. Granada and G. Martinez-Lopez, *unpublished*). Until recently, machismo disease was believed to be confined to the Cauca Valley of Colombia.

In this paper, we report the discovery in 1982 of soybean plants showing symptoms similar to those of machismo at the Mazatán Experiment Station near Tapachula, Chiapas, a region in southwestern Mexico where soybean production is rapidly expanding. We report evidence supporting the hypothesis that this disease is machismo and suggest that it may be a threat to soybean production well beyond the borders of Colombia.

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MATERIALS AND METHODS

Collection of plants. In November 1982, commercial plantings and experimental plots of soybeans in extreme southwestern Mexico were examined for the presence of plants showing symptoms similar to those reported for machismo in Colombia. Diseased plants of the Mexican cultivar UFV-1 were found at the Rosario Izapa Agricultural Experiment Station of the National Institute for Agricultural Research, Mazatán, near Tapachula, Chiapas, Mexico. Plants were photographed in the field and an estimate of the percentage of infected plants was made. Infected and healthy plants were removed from the field, soil was washed from the roots, and the root area was enclosed in a plastic bag containing moist vermiculite and small amounts of captan and aldicarb (Temik) (as requested by the Agricultural Plant Health Inspection Service for a permit for import of diseased plant material) for transport to the University of Illinois at Urbana-Champaign. Soybean plants identified as machismo-diseased or healthy were collected in a similar manner from fields in the Cauca Valley of Colombia. In Urbana, plants were maintained under quarantine in a growth chamber at 24 C on a 16-hr light photoperiod regime.

Collection and curation of insects. Quarantine regulations did not permit import of live insects into the United States. A small sweep net was used to collect insects from affected fields in Mexico. The catch was preserved in 70% ethanol and transported to Urbana for preliminary sorting and identification by J. K. Bouseman. The leafhoppers from the sweepings of infected plants were sent to the USDA Systemic Entomology Laboratory, Smithsonian Institute, Washington, DC, where they were identified by J. P. Kramer. Voucher specimens were deposited in the International Soybean Arthropod Collection at the Illinois Natural History Survey at Champaign.

Dienes' staining. Within 2 days of the removal of plants from the field in Mexico, Dienes' staining (5), a technique to detect MLOs in plant tissue, was attempted on hand-cut sections of stems and petioles of diseased and apparently healthy soybean plants.

Preparation of samples for electron microscopy. Within 3 hr of the removal of diseased and healthy soybean plants from the field, 1-mm sections of petioles, midribs, and stems were placed at room temperature in 4% glutaraldehyde in a buffered sucrose solution (0.1 M sodium phosphate, pH 7.1, and 0.2 M sucrose). The sections were fixed overnight, then rinsed and stored in the same buffered sucrose solution without glutaraldehyde for transport to the United States. Postfixation was in 1% OsO_4 in buffered sucrose for 2 hr; block staining was in 1% aqueous uranyl acetate for 3 hr at room temperature. Samples were dehydrated in ethanol, immersed in propylene oxide, and embedded in Epon 812. Sections 80–150 nm thick were cut with a glass or diamond knife on an LKB ultramicrotome and poststained with uranyl acetate and lead citrate.

Attempts to isolate a cultivable MLO or spiroplasma. Young leaf, stem, and

pod tissue was removed from infected and healthy plants from Mexico and Colombia and minced in 5 ml of DSM4 (4) medium. After 10 min of incubation at room temperature, the medium was filtered (0.45 μm), and 0.1 ml was transferred to 5 ml of LD8 liquid medium (3). An additional 0.1 ml of filtrate was plated on LD8 agar. Tubes and plates were incubated at 31 C. Liquid cultures were checked by dark-field microscopy, and plates were examined through a dissecting microscope at frequent intervals for 4 wk.

Comparison of virescence symptoms in Mexican and Colombian soybeans. Virescence, a symptom seen in Mexico, had not been reported in machismo-diseased soybeans from Colombia. In a comparative study in Colombia, leafhoppers (*S. fuliginosus*) were obtained from a colony that was originated from insects collected from machismo-affected fields. Adult leafhoppers were caged in groups with three to five infected plants for acquisition access. Inoculative insects were then caged in groups of 10–20 on 12 soybean seedlings (cultivar UFV-1 or ICA-Tunía) 5–10 days old. Inoculation access periods were 5–10 days. Control plants of both cultivars were not exposed to insects. Plants were observed for development of virescence from the time of blooming and pod formation until plants showed characteristic witches' broom symptoms of machismo.

RESULTS

Symptomatology. Mexican soybean plants with machismo-like symptoms showed delayed senescence, retaining their leaves, green color, and some flowers. Unaffected plants were at full maturity and were brown, defoliated, and partially dry. Disease incidence in the field was less than 0.2%. Diseased plants were characterized by proliferation of small branches and deformed pods at lateral nodes, and some plants had phyllody and virescence of floral petals (Fig. 1). Pods on infected plants ranged from normal to diminutive pods oriented in an upright position (Fig. 2) to corrugated, leaflike structures (Fig. 3) that sometimes enclosed tiny, undeveloped seeds and sometimes were merely curled but not joined and lacked seeds. These symptoms agreed with those of the 1979 report of Colombian machismo, with the exception of virescence.

After soybean plants in the greenhouse in Colombia (UFV-1 from Mexico and ICA-Tunía from Colombia) were exposed to *S. fuliginosus* carrying the Colombian MLO, typical machismo symptoms were observed in both groups. Virescence, observed in both cultivars, was expressed late in disease development when plants appeared to be severely infected. Leaves and petals were of similar size and color, although petals had pale white edges. Flowers were often located within witches' broom proliferations and were identifiable only because of the presence of anthers. From such flowers, closed, corrugated leaflike structures formed instead of pods. Cultivar UFV-1 was as susceptible to the Colombian machismo agent as cultivar ICA-Tunía under these greenhouse conditions.

Tests of seed transmission from field-collected, infected plants were unsuccessful because seeds from infected pods did not germinate. All attempts to graft- or dodder-transmit the pathogen failed, probably because plants were senescing and near death.

Insects. Thirty-eight leafhoppers (Homoptera: Cicadellidae), representing four genera, were swept from one diseased soybean field at Mazatán, Chiapas. Of these specimens, 18 (47%) were *S. fuliginosus*, 17 (45%) were *Agallia lingula* (Van Duzee), 2 (5%) were *Balclutha hebe* (Kirkaldy), and 1 (3%) was *Ponana* sp. Only *S. fuliginosus* has been reported to transmit the causal agent of machismo disease.

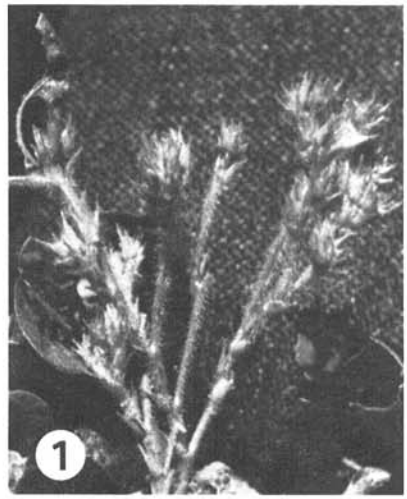
Dienes' stain. Two of three diseased soybean plants from Mexico showed darkened blue areas of stained cells in the phloem region of stems and petioles when examined with light microscopy. No stained areas were found in symptomless plants from the same field.

Electron microscopy. MLOs (Figs. 4 and 5) were found in samples from three of three machismo-diseased soybean plants from Mexico and in samples from two of two machismo-diseased soybean plants from Colombia. Many of the observed phloem sieve elements were infected with numerous MLOs that were morphologically indistinguishable in samples from the two countries. The morphology of the MLOs included circular, filamentous, and pleomorphic profiles in sections varying in electron density (Fig. 4). No helical forms were observed. The ultrastructure consisted of ribosomes, a net of presumed DNA fibrils, and a single plasma membrane without a cell wall (Fig. 5). No viruslike particles or other plant pathogens were observed.

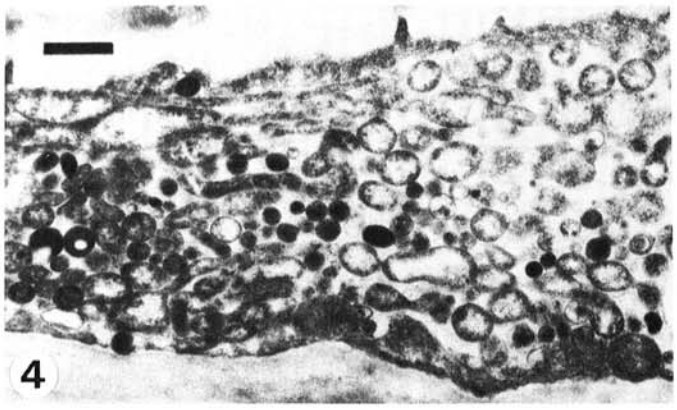
Cultivation attempts. No organisms were found in broth or semisolid media from either diseased or apparently healthy plants.

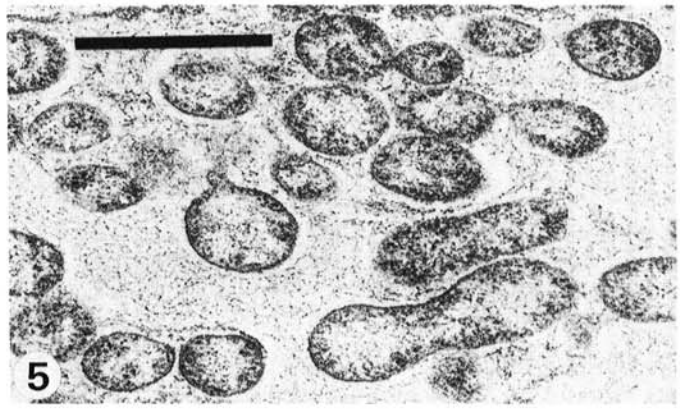
DISCUSSION

Symptoms of the diseased Mexican soybeans closely resemble those described for machismo disease in Colombia (7). Although Granada did not originally report virescent flowers in Colombian soybeans from the field, the machismo agent did cause virescence when transmitted to bean (9) and to *Catharanthus* (*Vinca*) *roseus* (G. A. Granada, *unpublished*). The observation in this study that the Colombian MLO causes virescence in both UFV-1 and ICA-Tunía in the greenhouse removes doubt about symptom similarity. The MLOs found in machismo-diseased soybean plants from Mexico and Colombia in our study are similar in morphology, ultrastructure, and type of host cell infected to MLOs associated with many plant diseases as presumptive causal agents (2).



Figs. 1–3. Photographs of Mexican soybean plants showing machismo-like symptoms. **(1)** Virescent flowers of diseased plants; petals are green. **(2)** Diminutive, upturned pods of infected plants. **(3)** Corrugated, leaflike pods of infected plants.





Figs. 4 and 5. Electron micrographs of sections of a machismo-diseased soybean plant sample from Mexico showing mycoplasmalike organisms (MLOs). **(4)** Phloem sieve element containing many MLOs varying in morphology and electron density. **(5)** Higher magnification of MLOs showing the ribosomes, net of presumed DNA fibrils, and single, wall-less plasma membrane typical of plant-infecting MLOs. Scale bars = 0.5 μm.

Proof of pathogenicity and of relatedness of the Mexican and Colombian pathogens depends on the culture and characterization of the machismo-associated MLOs as it does for other plant-infecting MLOs. Direct comparison of disease and pathogen in host plants grown in the same environment was prevented by the quarantine prohibiting our use of vectors in the United States. Although no helical forms of MLO were observed in our study or isolated from diseased tissue, we cannot exclude the possibility that our data represent a nonhelical condition or aspect of spiroplasma. Our survey of diseased tissue by electron microscopy was limited and dark-field light microscopic examinations were not attempted.

The data on symptomatology, Dienes' stain, electron microscopy, and presence of the only reported vector of machismo in affected fields in Mexico provide strong circumstantial evidence to support the hypothesis that the disease in both cases is caused by the same or a similar MLO. If this is so, this report expands the reported geographical range of the machismo agent northwestward by about 2,500 km.

Derrick and Newsom (6) recently showed an MLO to be associated with soybean proliferation ("Q") disease, transmitted by *S. acutus* (Say) in the southern United States. Soybeans in South America, Africa, and Asia have been plagued with diseases characterized by witches' brooming and rosetting (1,10–13); although the causal agents are unknown, they are suspected to be of MLO etiology. Questions of possible relatedness of these agents to the machismo agent remain to be resolved.

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